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Radegen Biotechnology

Creative Work: RadegenBio+ (Formally Theta+) *de novo* stepwise DNA synthesis bead bound ssDNA substrate and bead bound dsDNA substrate concept.

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Bead Bound DNA Substrate – a ssDNA oligo nucleotide or a ssDNA synthetic DNA fragment that is tethered to a microparticle via a 5' end covalent bond. The oligo is composed of a defined and functional sequence. The microparticle can be composed of either a material that provides additional function aside from serving as a solid support or of a material that exclusively serves as a solid support. Specifically, the microparticles can be a magnetized particle or a plastic particle. The magnetized particle provides the additional function of particle isolation by magnetization using standard laboratory equipment. The plastic particle acts as a solid support for use in column-based purification methods. Both types of particles are coated with streptavidin. Both methods can and are used in the Radgenbio+ system.

For tier one assembly, a chemically synthesized oligo is bound to a microparticle substrate via a 5' linked biotin functional group. The oligo is of a defined sequence that provides several functions. The length contributes to optimum conditions that prevent steric hindrances and the sequence identity act as binding regions for DNA binding proteins with specific functions. A synthetic DNA fragment is produced by the 5' – 3' stepwise addition of nucleotides in a defined order to produce a construct of a defined sequence. After the final nucleotide in a sequence is added, an additional functional sequence can be added for downstream use. A synthetic dsDNA preparation is made by protection PCR.

For Tier two assembly, a ssDNA fragment is bound to a microparticle via a 5' biotin linkage. The fragment is universal and has functional sequences that help produce a circularized DNA molecule. The ssDNA fragment is then converted to a bead bound dsDNA library. Tier 1 products prepared by protection PCR have one of the ends modified with a 5' end inverted nucleotide to exclude the modified end in a ligation reaction. Next, a ligation reaction is conducted with tier 1 product with a sequence identity of a unique DNA construct that is meant to be adjacent to a vector sequence. Only the end of the dsDNA fragment lacking the protection group will ligate to the bead bound synthetic DNA fragment. The results from this reaction is a bead bound DNA prep that consists of the original bead bound fragment and the first tier 1 product. The final construct is developed by the step wise ligation of tier one products in a sequential order to maintain the sequence identity of a desired construct. The next ligation step occurs after a simple deprotection step that involves washing away the non-tethered antisense strand by NaOH treatment followed by a PCR step to produce a bead bound dsDNA prep with a reactive end.

Oligo Synthesis can also be accomplished by the preparation of a synthetic DNA fragments via a 3' biotin linkage of sense strand. The most 5' end of antisense strand is composed of the first nucleotide of a desired primer and the 3' end contains functional sequences for generating a pure ssDNA primer preparation by nicking the antisense non-tethered strand followed by NaOH treatment to melt the antisense strand from the bead bound DNA. The washed away ssDNA molecule consists of a sequence for use as a primer.

A primer independent polymerase is used in several steps of this process.